Annals of Nuclear Medicine Vol. 21, No. 1, 79–83, 2007

Reducing renal uptake of ^{111}In-DOTATOC: A comparison among various basic amino acids

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Purpose: Several studies have reported significant renal toxicity after the use of a high dose of ^{90}Y-DOTATOC. Thus, renal protection is necessary in treatments with ^{90}Y-DOTA Tyr3-octreotide (DOTATOC). The infusion of certain positively charged amino acids has been shown to effectively reduce renal uptake of DOTATOC. In this study, we compared the effectiveness of three kinds of amino acids, D-lysine (lysine), L-arginine (arginine) and histidine, on renal protection in healthy rats and tried to determine which one was the most effective. ***Methods:*** Twenty SD healthy male rats were divided into 4 groups: lysine, histidine, arginine, and control. The rats were injected with a dose of 400 mg/kg of amino acid or 2 m*l* of phosphate-buffered saline (PBS) (as control) intraperitoneally. All rats were sacrificed at 4 hrs after the injection of 1 MBq ^{111}In-DOTATOC. Samples of the kidney were taken and weighed carefully. The counts of radioactivity were measured by a gamma counter and renal concentrations were calculated and expressed as percent injected dose per gram (% ID/g). ***Results:*** The renal uptake of ^{111}In-DOTATOC was significantly lower for all three kinds of amino acids when compared to the control group. The renal uptake of ^{111}In-DOTATOC in the lysine group was significantly lower than those in the histidine and arginine groups. The renal uptake of ^{111}In-DOTATOC in the histidine group was lower than that in the arginine group, but no statistical difference was noted. ***Conclusion:*** Among these three amino acids, lysine had the best reduction rate of renal uptake of DOTATOC. Histidine was more effective than arginine but no statistical difference was noted.

Key words: DOTATOC, indium-111, lysine, histidine, arginine

INTRODUCTION

RADIOLABELED somatostatin analogs have proven helpful in the diagnosis and treatment of neuroendocrine tumors which contain somatostatin receptors.[1–3] ^{111}In-DTPA pentetreotide (octreoscan) has demonstrated a good accuracy rate in the diagnosis of these tumors.[4–6] Surgery is the primary form of treatment, but in most patients surgery cannot be curative because of metastatic spread at the time of diagnosis. Moreover, chemotherapy rarely cures patients, so alternative modes of therapy are needed. Octreoscan with the Auger and conversion electron emission of ^{111}In has been shown to be effective in the treatment of neuroendocrine tumors.[7] However, ^{111}In is not an ideal radionuclide for therapy. For therapeutic purposes, yttrium-90 (^{90}Y) is considered one of the best radionuclides due to its favorable physical and chemical characteristics. Recently, newer therapeutic approaches with the use of the beta-emitter yttrium-90 conjugated via DOTA to Tyr3-octreotide (DOTATOC) have been

Received May 29, 2006, revision accepted October 24, 2006.
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developed.[8]

In a pilot study, tumor response and symptomatic relief were achieved with ^{90}Y-DOTATOC treatment of a patient with somatostatin receptor-positive metastases of a neuroendocrine carcinoma.[9] In subsequent studies, the results were promising and convincing.[8,10–12] However, in several other studies, significant renal toxicity was found after the use of a high dose of ^{90}Y-DOTATOC.[10,11,13,14] Renal protection is thus necessary in the treatment with ^{90}Y-DOTATOC. The infusion of certain amino acids, particularly D-lysine (lysine) and L-arginine (arginine), has been shown to block renal tubular peptide reabsorption.[15–17] In this study, we compared the effectiveness of various amino acids on the reduction of renal uptake of ^{111}In-DOTATOC in healthy rats and tried to determine which amino acid was the most effective for renal protection in this treatment.

MATERIALS AND METHODS

1. Experimental animals

Sprague-Dawley (SD) healthy male rats (200–250 g) were used in the experiment. Rats were housed in a temperature-controlled room (25°C) with daily artificial illumination. Food and water were given *ad libitum*. This study was approved by the local Institutional Review Board based on the Helsinki recommendations and internationally accepted principles for the care and use of experimental animals. The animal care was in accordance with the Guide for the Care and Use of Laboratory Animals in Taichung Veterans General Hospital.

2. Preparation of ^{111}In-DOTATOC

The preparation of ^{111}In-DOTATOC followed the procedure described previously.[18,19] Briefly, 20 μg of DOTATOC (Institute of Nuclear Energy Research, Lungtan, Taiwan) was dissolved in 50 μl sodium acetate buffer (0.4 M, pH 5.5) with 1 mg gentisic acid; after the addition of 222 MBq (6 mCi) $^{111}InCl_3$ (0.05 M HCl), the solution was heated at 90°C for 25 min. Quality control was obtained with the use of Sep-Pak C18 cartridge and high-performance liquid chromatography (HPLC) as previously described.[18] The radiochemical purities of ^{111}In-DOTATOC were more than 95%.

3. Preparation of amino acids

Three kinds of amino acids, lysine, histidine and arginine (all from Sigma Chemical Company, Dorset, UK), were used in this study. Each amino acid (2.5 g) was diluted with 50 m*l* of phosphate-buffered saline pH 7.2 (PBS) and filtered with 0.22 μm filter membrane. The solution with a concentration of 50 mg/m*l* was ready for injection.

4. Biodistribution

Twenty SD male healthy rats were divided into 4 groups (5 rats in each group): a lysine group, histidine group, arginine group, and PBS (control) group. The rats were anesthetized with ketamine and injected with a dose of 400 mg/kg of the amino acid or 2 m*l* of PBS intraperitoneally. Thirty minutes later, the rats were intravenously injected with 1 MBq of ^{111}In-DOTATOC. All rats were sacrificed at 4 hrs after the injection of ^{111}In-DOTATOC. Samples of the kidney were taken and weighed carefully. The counts of radioactivity were measured with a gamma counter and tissue concentrations were calculated and expressed as percent injected dose per gram (% ID/g).

5. Statistical analysis

Statistical analysis of the results was made with the Statistica for Windows Release 4.5 package (StatSoft, Inc., OK, US) using Kruskal-Wallis ANOVA and Median tests and Mann-Whitney U tests. Results are expressed as mean ± S.T.D. A p value less than 0.05 was considered statistically significant.

Table 1 Effectiveness of various amino acids in reducing renal uptake after injection of ^{111}In-DOTATOC

Groups	Kidney (% ID/g)	Reduction rate of renal uptake
Lysine	1.52 ± 0.35	56.8%
Histidine	2.24 ± 0.55	36.3%
Arginine	2.45 ± 0.50	30.3%
Control	3.52 ± 0.77	

Table 2 Statistical results of the renal uptake (% ID/g) of ^{111}In-DOTATOC for the three amino acids groups using Mann-Whitney U tests

	Lysine	Histidine	Arginine	Control
Lysine	1	0.045	0.016	0.009
Histidine	0.045	1	0.464	0.028
Arginine	0.016	0.464	1	0.047
Control	0.009	0.028	0.047	1

RESULTS

The effects of various amino acids on the reduction of renal uptake after injection of ^{111}In-DOTATOC are shown in Table 1. The average renal uptake of DOTATOC was 3.52% ID/g in the control group and was reduced to 1.52, 2.24 and 2.45 in the lysine, histidine and arginine groups, respectively. Lysine was most effective with a reduction rate of 56.8%, followed by histidine with a reduction rate of 36.3% and arginine with a reduction rate of 30.3%. The statistical results of the renal uptake (%ID/g) of ^{111}In-DOTATOC for the three amino acid groups are shown in Table 2. The renal uptake of ^{111}In-DOTATOC was significantly lower for the three positively charged amino acids when compared to the control group. The renal uptake of ^{111}In-DOTATOC in the lysine group was significantly lower than those in the histidine and arginine groups. The renal uptake of ^{111}In-DOTATOC in the

histidine group was lower than that in the arginine group, but no statistical difference was noted.

DISCUSSION

^{90}Y-DOTATOC therapy has proven to be an effective treatment for patients affected by tumors expressing SST receptors.[8,12,13] Although these preliminary results are encouraging, some severe complications such as renal toxicity were noted accompanying the treatment. Recent biodistribution studies showed that the highest predicted absorbed doses to normal organs were to the kidneys and spleen.[20,21] Since no spleen toxicity has ever been found after the therapy, it is clear that the kidney must be the dose-limiting organ in receptor radionuclide therapy with ^{90}Y-DOTATOC. Sporadic cases of delayed renal failure have been observed, especially in patients who have received >7.4 GBq/m^2.[8,10] Cybulla et al. reported a case of a 78-year-old woman with a carcinoid of the small intestine with multiple metastases in the liver. Fifteen months after the ^{90}Y-DOTATOC therapy, a progressive deterioration of renal function occurred, leading to end-stage renal disease.[10] The optimal dose of ^{90}Y-DOTATOC can not be achieved in some patients due to the risk of renal toxicity. Therefore, reducing kidney uptake is critical in the treatment with ^{90}Y-DOTATOC.

Infusion of positively charged amino acids has been reported to effectively reduce the renal uptake of these radio-peptides.[15–17,22] In these studies, lysine and arginine were the most commonly used amino acids. Histidine is also a positively-charged amino acid, and should have an effect on the reduction of renal uptake of DOTATOC. To the best of our knowledge, there have been no reports on the effect of histidine on the inhibition of renal uptake of DOTATOC and no comparison has been made among these three amino acids. According to our results, all three amino acids significantly inhibited renal uptake of DOTATOC when compared to the control group, and lysine was the most effective. Histidine was slightly more effective than arginine in reducing renal uptake of DOTATOC. However, no statistical significance was noted. Recently, Rolleman et al. reported that a combination of 25 g lysine plus 25 g arginine induced a 33% ± 23% renal dose reduction.[23] The combination of lysine and arginine but not lysine alone may be more effective in reducing renal uptake of DOTATOC. We suggest that a combination of lysine and histidine may merit further study since histidine was also effective in reducing renal uptake of DOTATOC in our study.

According to the study by Bernard et al., both D-lysine and L-lysine could result in significant inhibition of kidney uptake of ^{111}In-DTPAOC. However, ^{111}In-DTPAOC was decreased not only in the kidneys but also in somatostatin receptor-positive organs such as the pancreas and adrenal glands after L-lysine administration. In contrast, D-lysine did not have such an effect in octreotide receptor-positive organs. They concluded that D-lysine may be preferred to L-lysine for reducing renal uptake of radioactivity because it does not interfere with the natural amino acid metabolic balance.[17] Therefore, we chose D-lysine instead of L-lysine for our study. In addition, in their study, the administration of D- or L-lysine in a single intravenous dose of 400 mg/kg resulted in obvious inhibition of kidney uptake of ^{111}In-DTPAOC at all time points tested, independently of the mass of ^{111}In-DTPAOC used. Higher or repeated doses of lysine did not give a significantly higher percentage inhibition.[17] The dose of 400 mg/kg of lysine was also proven to be effective in reducing renal uptake of radiolabeled peptides in other studies.[22,24] Therefore, we chose a dose of 400 mg/kg for the evaluation of these amino acids in our study.

The amino acids can be given orally, intravenously or intraperitoneally. All three administration routes have been investigated and proven effective in reducing the renal uptake of radiolabeled peptides in the literature.[17,25] In the study by Pimm et al., lysine was injected intraperitoneally and significantly reduced the renal uptake of ^{111}In labeled Fab fragment in mice.[25] Bernard et al. found both intravenous and intraperitoneal administration of lysine had an obvious inhibitory effect on the kidney uptake. Although the inhibitory effect of intravenous administration was greater than that of intraperitoneal administration, no statistical significance was found.[17] We chose intraperitoneal injection for the administration of amino acids because intraperitoneal administration is easier to perform than intravenous injection in rats. In addition, intraperitoneal administration is associated with a lower incidence of extravasation and leakage, which often occur in a small rat model and may lead to an unreliable result.

Several factors could be involved in renal toxicity, among which absorbed dose to kidneys likely plays a role. The radiolabeled peptide is rapidly cleared from the circulation by the kidneys and, after filtration by the glomerular capillaries, is subsequently reabsorbed almost completely (>99%) by the proximal tubular cells. The mechanism of the reduction of the renal uptake of radiolabeled peptides seems to depend on an inhibition of the tubular reabsorption.[13] Membranes of renal tubular cells contain negatively charged sites, to which positively charged amine or guanine groups of peptides can bind.[7] Thus, decreased binding of DOTATOC after administration of the positively charged amino acids can be explained by this phenomenon.[9] The interaction between positively charged peptides and negatively charged membranes of renal tubules has previously been documented by Akizawa et al.[26] In their study, they used an octreotide with a negative charge on the *N*-terminal amino acid and effectively reduced the renal accumulation of radioactivity. They considered that this effect may be attributed to the reduction of lipophilicity and also the repulsive force rising from the negatively charged renal brush border membrane.

The use of highly concentrated amino acid solutions has many adverse effects.[12,27] Nausea and occasional vomiting have been frequently reported. L-Arginine is mainly responsible for the gastrointestinal toxicity. This amino acid reduces the lower esophageal sphincter pressure and inhibits gastric emptying, either by the production of nitric oxide or a direct effect on the central nervous system.[28] In the study by Bodei et al., nausea and vomiting occurred in 50 and 69% of patients depending on the concentration of arginine in the solution, whereas only 10% of patients had gastrointestinal symptoms with lysine infusion alone.[12] Furthermore, hyperkalemia has been reported in patients receiving a high dose amino acid solution, particularly ketogenic amino acids, such as lysine.[12,27] The increased kalemia is probably due to an extracellular shift of potassium secondary to an increased production of ketonic bodies in an acidic environment.[27] In the study by Rolleman et al., profound hyperkalemia occurred in 50% of the patients infused with 75 g of lysine. The potential risk of cardiac arrhythmia, although rare, needs to be kept in mind when large amount of lysine is used and ECG monitoring is often necessary. Compared with lysine and arginine, side effects of histidine seem to be infrequent and less severe. In an animal study by Kasaoka et al., histidine decreased the food intake in the studied animals by activating histamine neurons, since histidine is a precursor of histamine.[29] Rabbani et al. found no safety concerns for L-histidine in their study and suggest that histidine is safe for human consumption.[30] However, histidine was administered orally in these studies. There is no available information regarding the side effects of high dose histidine, administered intravenously in a short period. Further study is necessary before drawing a conclusion about the safety profile of high dose histidine.

CONCLUSION

All three amino acids, lysine, histidine and arginine, were effective in reducing the renal uptake of DOTATOC when compared to the control group and lysine was the most effective. Histidine was more effective than arginine, but no statistical difference was noted.

ACKNOWLEDGMENTS

This study was supported by the National Science Council, Republic of China (Grant No. NSC 93-2314-B-075A-008).

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